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EFFECTS OF PRESSURE AND TEMPERATURE ON THE EKG AND HEART RATE OF THE HYDROTHERMAL VENT CRAB *BYTHOGRAEA THERMYDRON* (BRACHYURA)

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Abstract

Effects of pressure and temperature on the electrocardiogram (EKG) and heart rate of crabs from the Galapagos deep-sea (2500 meters) hydrothermal vents were studied. Vent crabs require high hydrostatic pressure for long term survival. During decompression their EKG is disrupted and their heart rate is reduced. Low temperature reduces these decompression effects. The crabs have a higher temperature tolerance while at their environmental pressure (238 atm) and can withstand short-term exposure to temperatures as high as 37°C. Possible mechanisms for the action of pressure on neuromuscular systems are discussed. Habits and physiological capabilities of the crabs in the unusual vent environment are suggested on the basis of their physiological tolerances.

Introduction

More than 89% of the ocean bottom is at depths greater than 1000 meters. Yet, so little research has been done in the deep-sea that hydrothermal vents with their associated biological communities have only recently been discovered (Corliss *et al.*, 1979). The vent habitat, at a depth of 2500 meters, is characterized by high pressure, high biomass and variable, high temperatures. This contrasts with the bulk of the deep-sea which is also characterized by high pressue but has low biomass and stable low temperatures. By comparing aspects of the physiology of vent species and species adapted to the "typical" deep-sea it is possible to gain insight into the relative importance of certain environmental factors in selecting for physiological characteristics of deep-sea species. In addition, the influence of physiological characteristics on the distribution of deep-sea species can be studied.

This study investigates the adaptations of the hydrothermal vent crab, *Bythograea thermydron*, to the high pressure and fluctuating temperatures (Corliss *et al.*, 1979; Speiss *et al.*, 1980) of the vent environment to determine how these adaptations might affect the crabs' distribution in the deep-sea. This species is predominantly found in the mussel and pogonophoran beds in the warm vent water (up to 22°C, anoxic, up to 300 μM H_2S/l) but, they are found also at the periphery of vent areas where the water temperature is 2°C (110 μM O_2/l, no H_2S). Although few data are available on the temperature tolerance of other deep-sea species, the general belief is that they tolerate a range of only a few degrees (George, 1979a; Childress *et al.*, 1978).

Many studies have examined high pressure effects on shallow-living species (Rice, 1961; Bayne, 1963; Knight-Jones and Morgan, 1966; Macdonald, 1972;

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Wilcock *et al.*, 1978) but few have examined pressure effects in deep-sea species. In studies on deep-sea species, decompression caused reduced activity and loss of coordination, which are reversed by recompression (George, 1979a, b; Macdonald and Gilchrist, 1978; Menzies *et al.*, 1974; Yayanos, 1978). These studies suggest that disruption of nerve functions occur in some deep-living species when they are decompressed. The only study of pressure effects on the neuromuscular system of a deep-sea crustacean indicates that pressure effects occur below as well as above the species environmental pressure (Campenot, 1975).

We chose to study the electrocardiogram (EKG) and heart rate of *Bythograea thermydron* as indicators of pressure effects on the neuromuscular system. This approach was chosen for two reasons. First, if pressure affects either the heart innervation or the heart muscle itself this would be indicated by changes in the EKG or heart rate. Secondly, the technique could be done inside a steel pressure vessel and under the constraints imposed by operating aboard ship. All of the experiments in this report were carried out at sea, aboard R.V. Gilliss and R.V. New Horizon, during two expeditions to the Galapagos vents study sites (January–February and November–December 1979).

Materials and Methods

Collection of specimens

Specimens of *Bythograea thermydron* were collected by the submersible *Alvin* from two vent areas: Mussel Bed (00°48.3'N, 86°09.1'W) and Rose Garden (00°48.9'N, 86°13.3'W). Baited traps were deployed and recovered by *Alvin* to capture the crabs. The traps were brought to the surface in an insulated container which protected the crabs from temperature changes but not pressure changes. The container was a 26 cm length of polyethylene pipe with an inside diameter of 30 cm and an outside diameter of 36 cm. The top and bottom were 5 cm thick polyethylene. A magnetic latch held the hinged top closed during ascent.

During the first expedition to the Galapagos hydrothermal vents, the captured crabs were kept at various temperatures and pressures because the environmental conditions permitting survival of this species were unknown. We changed our animal maintenance techniques as we gained information about the crabs requirements. The information collected during the first expedition showed us how to capture and maintain live crabs; during the second expedition we applied this knowledge in studies on the effects of pressure and temperature on their EKG and heart rate.

Methods and Results

First expedition

The crabs captured during the first expedition were either maintained at 1 atm and temperature of 2°, 7°, 10°, or 12°C or at 120 atm and 2°C. The crabs at 1 atm were kept in aerated 1 gallon (3.79 L) containers inside small refrigerators. Individuals at 120 atm were kept in a transparent pressure vessel through which aerated, chilled (2°C ± 0.5°C) seawater was circulated by a high pressure pump (Quetin and Childress, 1980). This apparatus allowed us to make behavioral observations at different pressures but was limited to a maximum pressure of 120 atm which was lower than the crabs' environmental pressure. These studies showed that individual *B. thermydron* survived longer at 120 atm than at 1 atm. The mortality rate at 1 atm was high; among 64 crabs the maximum survival time was

5 days and it appeared to decrease with increasing temperature. In comparison, none of the 25 crabs kept at 120 atm and 2°C died during the remaining 21 days of the expedition. Behavior of the crabs differed at the two pressures. Their movements seemed spastic and uncoordinated at 1 atm and apparent loss of balance was common with many individuals falling on their backs with their legs splayed outward. In contrast, crabs at 120 atm were active and their behavior appeared similar to the behavior of crabs observed in the vent environment from the submersible. The low pressure effects on behavior were reversible by recompression. Furthermore, repeated decompression for short time periods, had no apparent long-term effect. One of the crabs survived for 18 months in our laboratory in a pressure vessel at 238 atm and 5°C, even though it was decompressed for 15 minutes every 10 days for feeding.

In summary, observations made during the first expedition indicate that *Bythograea thermydron* requires high pressure for survival. It was also found that temperature influences the effects of low pressure and that neuromuscular function in this species is disrupted at low pressures.

Second expedition

Crabs collected during the second expedition were maintained at their environmental pressure (238 atm) and 5°C except when they were decompressed for use in experiments. We used a high pressure aquarium system similar to the one mentioned above, except constructed of stainless steel, to keep the crabs at this pressure. The pressure vessel had a volume of 16 liters and was surrounded by a temperature controlled water jacket.

The temperature tolerance of individual *B. thermydron* was determined at their environmental pressure. These experiments were conducted in a small (6 l) stainless steel pressure aquarium system. A unique protocol was developed for determining the crabs temperature tolerance because they were unable to withstand high temperatures while at low pressure. Individuals were decompressed from 238 atm to 1 atm (2 atm/sec) and transferred from the maintenance vessel to the experimental vessel while both vessels were at 5°C. The pressure was increased to 238 atm after sealing the experimental vessel. The temperature was kept at 5°C for one hour and then was increased to the test temperature (at 0.2° to 0.3°C/min), slowing as the test temperature was reached due to equipment limitations. After one hour of exposure to the test temperature, the temperature was again reduced to 5°C (0.1°C/min), the crabs were decompressed and the mortality determined. Five crabs were tested at each of the following temperatures: 30°, 35°, 37°, or 40°C. All of the crabs in the groups exposed to 30° and 35°C (5 at each temperature) survived, but none in the 37.5° or 40°C groups survived the one hour exposure. These results showed that the vent crab could withstand a one hour exposure to temperatures as high as 35°C while at their environmental pressure of 238 atm.

The effects of pressure and temperature on the heart rate and EKG of individual *B. thermydron* was determined by implanting electrodes in the pericardial cavity. These electrodes were 22 gauge teflon-coated silver wire with the teflon insulation removed from the last 0.5 mm. The technique for implanting electrodes is as follows. An animal was removed from the maintenance vessel, secured with rubber bands to a piece of plexiglass and placed in a pan of cold seawater (approximately 2°C). A small hole for the recording electrode was drilled through the carapace over the heart and a hole for an indifferent electrode was drilled posterior to this, near the edge of the carapace. Electrodes were cemented into each of the holes with dental

cement. This entire procedure took fewer than 15 minutes. The experimental crab, while still restrained, was placed in a pressure vessel at 5°C and the electrodes connected to electrical feedthroughs. The EKG signal was amplified and recorded on a high speed chart recorder.

Initial studies were performed to determine if the EKG and heart rate were stable after electrode implantation and if they were affected by the rate of compression and decompression. The short term effects of electrode implantation were investigated by recording the EKG for several hours immediately after recompression to 238 atm. The effects of compression and decompression were determined by recording the EKG during rapid changes in pressure. From these experiments we were able to determine the time course of the pressure effects.

The recordings showed no obvious short-term effects of electrode implantation. At 238 atm, the heartbeat was steady but the amplitude decreased with time (Fig. 1). Removal of the electrodes at the end of the experiments showed that material was deposited on them. This may have caused the amplitude decrease during the experiments by reducing the contact area of the silver electrodes with the body fluids. Neither a decompression rate of 24 atm/sec, nor a compression rate of 5 atm/sec affected the heart rate or the shape of the EKG form. When pressure effects were observed, they developed in fewer than 15 seconds after a change in pressure (Fig. 2).

To determine the effects of pressure and temperature on the EKG and heart rate of *Bythograea thermydron* the experimental animal was kept at 238 atm and 5°C for 30 minutes after electrode implantation and then subjected to a series of pressures in one of the two following orders: 238, 272, 340, 272, 204, 136, 68, 1, 68, 136, 204 and 238 atm or 238, 204, 136, 68, 1, 68, 136, 204, 272, 340, 272, and 238 atm. The maximum pressure of 340 atm was used because of the limitations

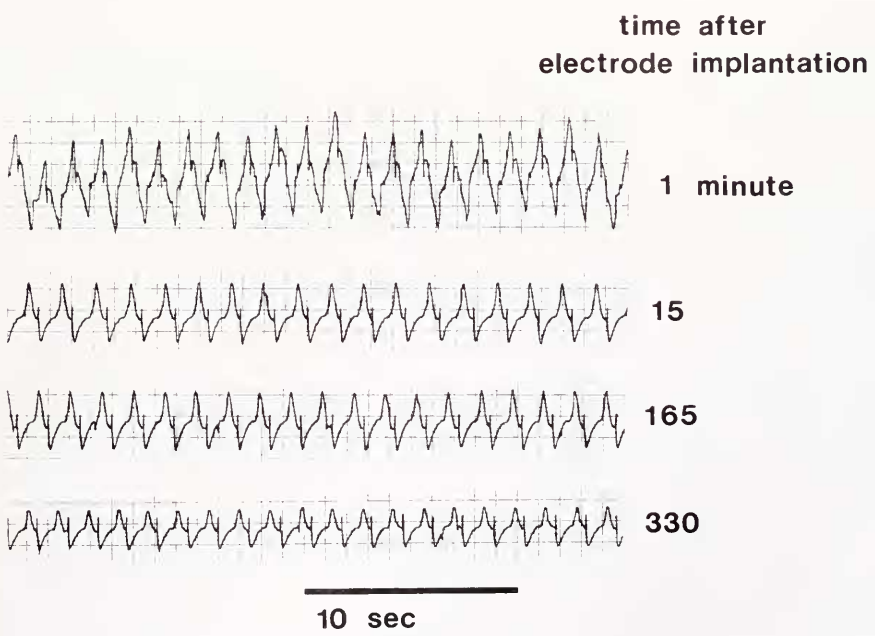

FIGURE 1. Time course for the stabilization of the EKG after electrode implantation in *Bythograea thermydron.*

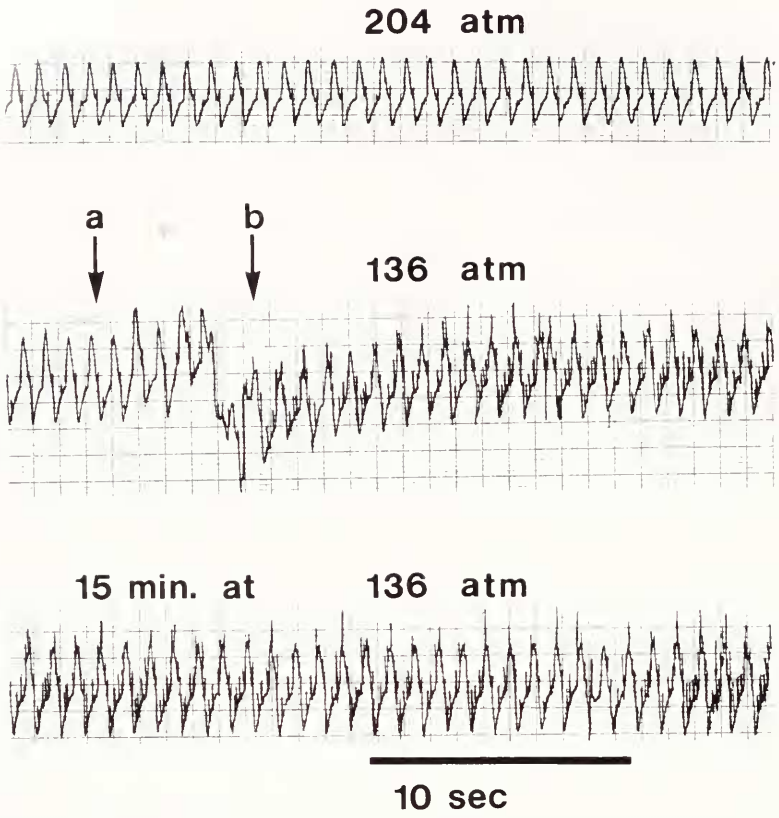

FIGURE 2. The time course for the development of the pressure effect in *Bythograea thermydron* at 12°C. a—immediately after decompression from 204 to 136 atm, b—development of the pressure effect (spikes overlying the normal EKG pattern).

of our equipment. Each crab was subjected to each pressure for 15 minutes during which the EKG was recorded for a 1 minute period every 3 minutes. Pressure changes were made at a rate of approximately 3 atm/sec. The crabs were tested sequentially at 3 temperatures, either in the order 5°, 12°, 20° or 5°, 20°, 12°C. Five individuals were examined.

Analysis of the EKG recordings emphasized changes in heart rate and EKG form. Changes in EKG amplitude were not considered in the analysis for two reasons. First, EKG amplitude was affected by the length of time that the recording electrodes were implanted in the experimental animal. Second, pressure caused small changes in the amplitude due to effects on the electrical connectors that penetrated the pressure vessel.

The results showed that the heart rate of the vent crab was not significantly ($P > 0.1$, t-test) affected by pressure over the range of 1 atm to 340 atm at 5°C (Fig. 3). At 12°C and 20°C the heart rate was significantly lower at 1 atm than at higher pressures ($P < 0.05$, t-test). Depression of the rate occurred at higher pressures at higher temperatures. At 12°C the heart rate slowed at pressures below 68 atm, while at 20°C it slowed below 136 atm. The form of the EKG trace also changed with pressure and temperature and showed interaction of these parameters.

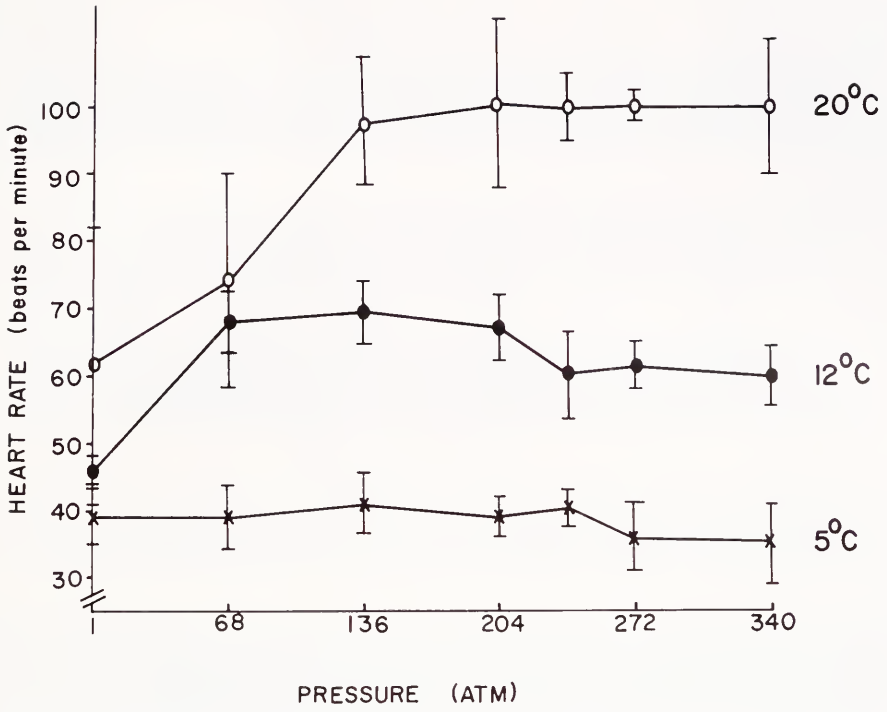

FIGURE 3. The effect of pressure and temperature on the heart rate of the crab, *Bythograea thermydron*. Error bars are ±1 S.E.

In this case, the pressure effect was characterized by random ($0.5 < P < 0.75$, goodness of fit test) spikes overlying the "normal" EKG (the EKG form at 238 atm) (Fig. 4). This pressure effect could not be attributed to an artifact of pressure and temperature effects on the recording electrodes because no electrical activity was recorded from electrodes placed in the pressure vessel alone or implanted in dead crabs exposed to the same experimental conditions. The disruptive effects of reduced pressure on the EKG form occurred at higher pressures at higher temperatures. At 5°C the disruption occurred at pressures between 1 atm and 68 atm, at 12°C it occurred between 68 atm and 136 atm and at 20°C it occurred at 136 atm to 204 atm. At the crabs environmental pressure of 238 atm, a temperature of 30°C disrupted the heartbeat (Fig. 5) and three of the five experimental animals died within two hours.

The time course for the development of pressure effects was not significantly different at the three temperatures tested (mean ± 1 S.E.: 5°C, 7.9 ± 1.61; 12°C, 9.00 ± 2.58; 20°C, 9.67 ± 1.20). These values were determined after a 68 atm drop in pressure and are the times from the end of the pressure change till the first appearance of at least 4 random spikes in each heartbeat. These are estimates for the time of development of pressure effects because it is unknown exactly when the effect began during the 15 second decompression. Further study will be required to determine the exact time course for decompression effects and also to determine the time course for the reversal of these effects during recompression. Reversal of decompression effects, however, appears to be dependent on time spent at low pressure.

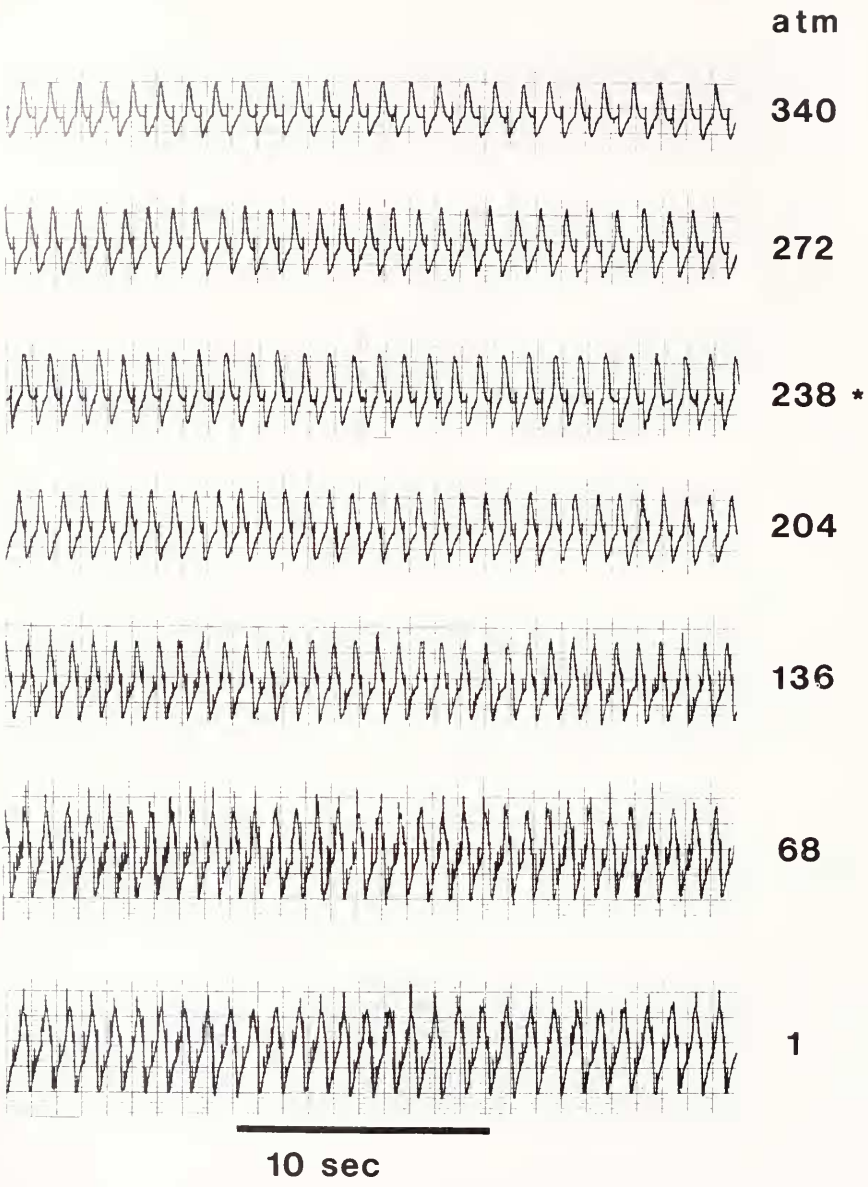

FIGURE 4. The effect of pressure on the EKG form of the crab *Bythograea thermydron* at 12°C; (*) the pressure normally experienced by the crab in its environment.

DISCUSSION

The disruption of the hydrothermal vent crabs electrocardiogram (EKG) during decompression is the first direct evidence of low pressure effects on the neuromuscular system of a deep-sea species. The increased electrical activity of the heart muscle is perhaps indicative of effects throughout the neuromuscular system which may account for the crabs abnormal behavior and eventual death at low pressures.

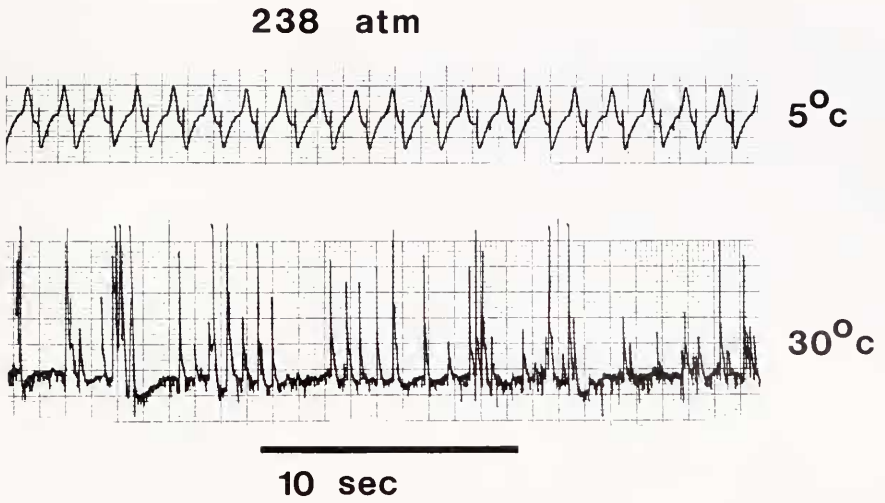

FIGURE 5. The disruptive effect of high temperature on the EKG form of *Bythograea thermydron.*

Deep-sea amphipods show similar behavior, indicative of neuromuscular effects, when they are decompressed. They display jerky pleopod movements and have difficulty in initiating pleopod rhythm when decompressed to half their environmental pressure (Yayanos, 1981).

Recent investigations provide evidence for several mechanisms by which pressure could affect the neuromuscular functioning of deep-living species. One mechanism may act through effects on synaptic transmission (Campenot, 1975). In the moderately deep-living (300–1600 meters) crab, *Geryon quinquidens*, high pressure depresses the excitatory junctional potential (ejp) amplitude in muscle fibers (Campenot, 1975). Excitatory junctional potentials reflect the local response of muscle membrane to transmitter released by nerve endings. Frequency of nerve stimulation is one of the controlling factors of ejp amplitude and increases in stimulation frequency lead to greater ejp amplitude through increases in transmitter release (Dudel and Kuffler, 1961; Frank, 1973). When ejp amplitudes are great enough, muscle fibers contract. Campenot (1975) attributes the high pressure depression of ejp amplitude in *Geryon* to interference with the release of transmitter substance. If the same mechanism operates in vent crabs then the reverse effect, produced by reduced pressure, might also occur. If this is the case, release of transmitter at low pressures would be increased and greater ejp amplitudes would occur for a given frequency of nerve stimulation. Under these conditions, some nerve impulses which produce subthreshold ejp amplitudes for muscle contraction at high pressure would produce larger ejp amplitudes and therefore muscle contraction at low pressures. This could be responsible for the increased electrical activity in the EKG recordings.

Another mechanism by which pressure may affect the neuromuscular system of vent crabs is through changes in cell membranes. Membrane structure changes could be responsible for permeability changes leading to changes in nerve excitability. Brauer *et al.,* (1980) have shown that deep-living freshwater amphipods go into negative ion balance at 1 atm and Johnson and Miller (1975) have shown that pressure affects the permeability of model membranes to some ions. In addition, pressure induced changes in nerve membrane conductance have been demonstrated

in both shallow (Spyropoulos, 1957; Wann *et al.*, 1979) and deep-living species (Campenot, 1975). The mechanisms by which these effects operate are not yet understood but one possibility is that pressure can cause viscosity changes and phase transitions in membrane lipids (Yayanos *et al.*, 1978). Since high pressure is expected to have an ordering effect on membrane bilayer structure (Boggs *et al.*, 1976) it seems reasonable that decreased pressure would reduce the ordering and lead to increased membrane conductance. Additional evidence that membrane lipids may be involved comes from investigations on enzymes that show nonlinear Arrhenius plots. Ceuterick *et al.* (1978) have shown that lipids are responsible for breaks in the plot of *Azotobacter* nitrogenase and that the temperature at which the break occurs increases with increasing pressure. The results of another study indicate that Na^+/K^+-ATPase activity decreases with increasing pressure (De Smedt *et al.*, 1979). Plots of the ATPase activity versus pressure show a breakpoint that increases with increasing temperature. This agrees with pressure shifts for melting transitions in phospholipids and aliphatic chains. De Smedt *et al.* conclude that an aliphatic chain melting process is involved in the pressure dependence of Na^+/K^+-ATPase.

It is possible that none of the mechanisms mentioned above are responsible for the observed pressure effects. Effects on reaction rates and enzyme catalytic properties due to pressure effects on molecular volume changes could explain the observed changes both in synaptic transmission and in ion distribution. For example, synaptic transmission could be affected by a change in the rate of association of transmitter substance with the receptor on the post synaptic membrane (Akers and Carlson, 1976). Ion balance would be influenced if membrane bound ion transporting ATPases were affected (Pequeux and Gilles, 1977; Goldinger *et al.*, 1978; Hall, 1979). In the vent crab, however, pressure effects appear to involve a structural change rather than a kinetic or equilibrium change. This hypothesis is supported by two results. First, the time course for decompression effects on the EKG are rapid; frequently less than 10 seconds. Second, temperature does not alter the time course of the effect. If reaction rates or equilibria were involved then the time course would be influenced by temperature. Results of investigations on enzymes of deep-living fish further suggest that pressure effects on enzymes do not cause the low pressure effects observed in the vent crabs. The enzymes of deep-living fish are relatively insensitive to pressure in comparison to those of shallow species (Siebenaller and Somero, 1979).

Pressure affects the vent crabs heart rate as well as EKG and may reflect effects on the cardiac pacemaker. Pressure effects on pacemaker cells have been demonstrated in some mammalian heart preparations (Örnhagen and Hogen, 1977) and also in the rhythmically firing cells of *Helix* (Wann *et al.*, 1979). The exact effects of pressure on the heart rate of deep-living species is unclear. Decompression causes an increase in heart rate in the deep-sea ostracod *Gigantocypris mulleri* (Macdonald, 1972) but in the mysid *Gnathophausia zoea* heart rate increases with compression. The influence of temperature on pressure effects on the heart rate of vent crabs is similar to that on the EKG. Lower temperatures reduce the effects of decompression.

The influence of temperature on pressure effects has been noted in other studies but in some cases the results conflict (Napora, 1964; Teal and Carey, 1967; Johnson and Eyring, 1970; Gillen, 1971; Childress, 1977; George, 1979b). This may be caused by several factors. First, pressure affects many levels of organization of biological systems and may affect some more than others depending on the species. Second, pressure effects may be increased, decreased or nullified by temperature

depending on the mechanisms by which pressure acts. Conflicting data on pressure effects also comes from early studies on shallow freshwater and marine organisms. Organisms were subjected to pressures far outside their normal range in these studies and little regard was given to temperature (Fontaine, 1930; Ebbecke, 1935; review by Gordon, 1970). In more recent investigations, pressures and temperatures within the environmental ranges of the species tested were used. These studies show that pressure generally has little effect (Teal and Carey, 1967; Pearcy and Small, 1968). When pressure effects occur, they are slight and are counteracted by low temperature (Napora, 1964; Teal, 1966, 1971). The experimental results on vent crabs agree with these latter studies. Although pressures below 68 atm apparently lead to disruption of the vent crabs neuromuscular functioning which eventually is lethal, no effects occur over the range of 136 to 340 atm. In general, vent crabs are only affected by pressures far outside their habitat range.

The temperature and pressure tolerance characteristics of vent crabs provide insight into their habits in the vent environment. The crabs high temperature tolerance and ability to live over a wide temperature range is extremely different from the limited temperature tolerance of other deep-sea species and species living at stable low temperatures in the antarctic (McWhinnie, 1964; Somero and DeVries, 1967; George, 1979a, 1979b). The crabs utilize this capability and are found throughout the vent environment. They have been observed in the warmest water exiting the vents (22°C) and also at the periphery of the vent environment where the temperature is 2°C. Their ability to withstand low temperature implies that they may be able to escape a dying vent or one showing increased thermal activity by leaving the vent habitat. The observed distribution of greater numbers of crabs in the warm water, therefore, probably reflects ecological factors and not physiological limits. The greatest biomass and consequently the greatest amount of potential food is near the warm water. Vent crabs have been observed eating pieces of vestimentiferan worms, which are most abundant in the warm water. Unlike vent crabs, distributions of other species around the vents may be influenced by the high temperatures. Most vent species appear to be endemic and relatively few non-vent deep-sea species are found near the Galapagos vents. Non-vent species may be restricted by their temperature tolerances from taking advantage of the vent environments high biomass.

The distribution of vent crabs apparently is not limited by temperature but it may be influenced by pressure. They may be limited to depths greater than 680 meters due to disruption of their neuromuscular functioning at pressures below 68 atm. The crabs upper lethal pressure limit was not determined due to limitations of our equipment but no high pressure effects were observed at pressures up to 340 atm, suggesting that the crabs could live at depths of at least 3400 meters. However, it is important to note that a species pressure tolerance may not be a reliable indicator of their depth distribution. This is mainly because the relative importance of genetic factors versus acclimation on pressure tolerance is not yet understood. Studies show that individuals of the same species, collected from different depths, may have different pressure tolerances (George, 1979a). Also, the pressure tolerance of even shallow-living species can be slightly increased by acclimation to elevated pressures (Avent, 1975). The temperature and pressure tolerance characteristics of vent crabs suggest that they are not limited by these environmental parameters to the vent habitat. They also are not limited by a high food requirement because their metabolic rate is comparable to that of other deep-sea crustaceans (Mickel and Childress, in prep.). Since the crabs apparently are not restricted to the vent environment by their physiological characteristics, their distribution is

presumably the result of behavioral patterns evolved in response to selection by ecological factors.

These studies on the vent crab may provide insight into factors which result in the failure of many groups of animals to penetrate into the deep-sea. For example, Wolff (1961) states that of a total of approximately 3500 brachyuran crab species only about 125 are found at depths greater than 200 meters. Brachyuran crabs apparently are not limited by physical characteristics of the deep-sea because the vent crabs have evolved physiologically to live at high pressures and low temperatures. Since the metabolic rate of vent crabs at low temperatures appears to be comparable to other deep-sea crustaceans, (caridian shrimps) which as a group live to much greater depths, metabolic rate adaptation also does not appear to be a limit. It seems likely, therefore, that the failure of brachyuran crabs to extensively inhabit the deep-sea is not due to an inability to evolve the necessary physiological abilities, but rather may be attributed to ecological factors which select against the brachyuran body form in most of the deep-sea. This suggests that, in general, the depth limits of taxa in the deep-sea may not be set by an inability to evolve appropriate physiological characteristics, but rather by the failure of a particular body form to be effective in the ecological milieu of the deep-sea.

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